Vol. XLIII. *October, 1922.* *No. 4.*

BIOLOGICAL BULLETIN

"REVERSAL OF INHIBITION" BY ATROPINE, IN CATERPILLARS.

W. J. CROZIER,

ZOÖLOGICAL LABORATORY, RUTGERS COLLEGE.

With a variety of invertebrate animals it has been found that strychnine exerts upon the neuromuscular structures an effect essentially similar to that which it displays in connection with the synapse-substance of vertebrate central nervous organs—namely, a decreased resistance to transmission, resulting in enhanced reactiveness; and a selective excitation of extensor elements, leading to opisthotonic curvature and to "reversal of inhibition" in responses involving reciprocal innervation. Representatives of the following groups are known to show behavior of this type under strychninization, and must therefore be accorded as a common property the possession of strychnine-receptive complexes suitably situated in their nervous organs: Platyhelminthia (*Planaria,*[1] *Bdelloura*[2]); Mollusca (*Chætopleura,*[3] *Chromodoris,*[4] *Limax,*[1] *Ouchidium,*[5] *Loligo*[6]); Echinodermata (*Thyone,*[7] *Asterias*[8]); Annelida (earthworm,[9] leeches[1]).

1 Personal observation.

2 Moore, A. R., *Jour. Gen. Physiol.*, I., p. 97, 1918.

3 Crozier, W. J., *Jour. Gen. Physiol.*, II, p. 627, 1921.

4 Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXIX., p. 261, 1919.

5 Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXXII., p. 443, 1921.

6 Moore, A. R., *Jour. Gen. Physiol.*, Vol. I., p. 505, 1919.

7 Pearse, A. S., BIOL. BULL., Vol. XVIII., p. 42.

8 Moore, A. R., *Jour. Gen. Physiol.*, Vol. II., p. 201, 1920; *Jour. Pharm. Exp. Therap.*, Vol. IX., p. 167, 1916.

9 Knowlton, F. P., and Moore, A. R., *Amer. Jour. Physiol.*, Vol. XLIV., p. 490.

Since strychnine has been relatively unique among neurophil substances in producing effects of the kind specified, it is desirable to call attention to the fact that among insects (caterpillars) phenomena identical in their nature with the reversal of inhibition typically induced by strychnine may be produced through the action of atropine; whereas, with these animals, strychnine is singularly ineffective and in fact fails to produce "reversal of inhibition." This point is important for the conception of progressive chemical differentiation of the nervous system in the animal series. Moreover, it raises certain difficulties in the way of employing strychninization as a test for the presence of synaptic structures or their homologues;[10] but further study is required to permit discussion of this aspect of the matter.

The experiments had in view the general impression that insects (and other arthropods) are peculiarly resistant to strychninization.[11] A common method of testing the action of a drug has been to submerge the organism in an appropriate solution, or to introduce the material into the alimentary canal. The cuticula of arthropods, however, may seriously interfere with such trials; its permeability is but little understood. Similarly, with *Ascaris* Schroeder[12] reported that immersion for three hours in 0.5 per cent. solution of strychnine was without effect; but some time ago I observed that characteristic squirming movements could be induced by injecting weak strychnine solutions into the interior of the body.

Resort was accordingly had to hypodermic injection. With small insects such procedure is impracticable; moreover, it was desirable to use forms in which the nervous system had not undergone great condensation. Suitable material was found in certain large caterpillars, of which five species were employed: *Protoparce celeus, Samia cynthia, S. promethia, Automeris io,* and *Ceratomia catalapæ*. The last named was not quite so well adapted to the injection as were the first four, upon which most of the observations were made. Half-cubic-centimeter volumes of solution

[10] Parker, G. H., "The Elementary Nervous System," Philadelphia, 229 pp. 1920.

[11] Biberfield, J., *Ergebn. Physiol.*, Jahrg. 17, p. 175; and the fact is well known to economic entomologists.

[12] Schroeder, W., *Arch. Exp. Path. u. Pharm.*, Bd. XIX., 290, 1885.

could be injected without material loss of fluid through the puncture; sealing the puncture with celloidin, after withdrawing the injection needle, was found unnecessary, and indeed undesirable, as leading to local irritation. Strychnine injection was usually made laterally at about the level of the first prolegs; the needle, pointing anteriorly, was thrust through the cuticule of an intersegmental constriction. It may be stated at once that the level of the injection appeared not to influence the outcome.

Upon the injection of strychnine sulphate in concentrations less than 1 : 100 no efforts could be detected, beyond a momentary loss of the ability to creep; this was not specific and could be induced by injecting distilled water or Ringer solution. *Protoparce celeus* and other forms were observed to drink as much as 0.8 cubic centimeter or more of 1 per cent. strychnine sulphate.

In *Automeris io* injection of 0.5 c.c. of 1 per cent. strychnine sulphate led to twitching contractions of the abdominal musculature, sometimes symmetrical, sometimes better developed at one side; the legs and prolegs were quiescent, nonreactive to stimulation, and the prolegs particularly were pressed together. After 4 minutes a pinched proleg retracted its terminal comb, but no further response was observed; the body musculature was very "flabby." At about 10 minutes subsequent to injection there appeared a phenomenon, only partially reproduced with the other species, which is the most closely related among those observed to the "typical" strychnine effect. With the animal on its side, the body may be bent somewhat, either dorsally or ventrally. If the caterpillar be given a slight ventral flexure, rhythmic twitchings appear in the dorsal thoracic zone, which serve to make the body curvature distinctly concave dorsally; whereas, with a dorsal curvature initially impressed, the caterpillar remains in that position, although the dorsal twitchings persist. The twitchings of the dorsal muscle bands likewise continue when the larva is picked up by its posterior spine.

The stimulation of the contraction of dorsal muscle bands is similar to the stimulation of dorsal extensor muscles in other groups, but further evidence of characteristic strychnine action is lacking. It is probably significant that *Automeris io* is distinctly the most reactive, most "irritable," of the caterpillars studied.

With the other species employed in the experiments this effect was not seen. In each form used, however, strychnine in saturated solution (volume 0.5 c.c.) produced temporary but violent convulsive movements, incoördinated trembling movements of legs and prolegs, followed by quiescence, and often a rather strong *ventral* flexure and paralysis of the appendages. Twelve hours was sufficient to permit full recovery from the effects of even such doses of strychnine. Experiments with various aquatic larvæ (especially of *Psephenus*), and upon crayfish, further point to the very slight toxic effect of strychnine, and likewise show the detectable tendency to produce opisthotonic curvature. Crayfish, even when holes have been punctured in the cuticula, presumably facilitating absorption of the drug, were found to live for 9 days in a strychnine solution originally 1 : 1000 and slowly evaporated during the nine days that the test continued. The backward swimming of the crayfish is impeded by the strychnine, while there is maintained an incessant forward creeping.

In contrast with this behavior of the strychninized arthropod neuromuscular system, in annelids we observe typical "reversal of inhibition," although in the earthworm[9] it is perhaps more difficult to demonstrate than is true with leeches. Several species of leeches have been studied in dilute strychnine sulphate, and for present purposes the following findings may be cited: there is pronounced contraction of the dorsal longitudinal musculature, relaxation of the ventral, while in response to local irritation the customary behavior of longitudinal, transversal, and dorsoventral muscles is completely reversed by the drug.

Atropine.

Injection of 1 per cent. atropine sulphate, 0.5 c.c., produced in all forms studied an abrupt loss of creeping ability, within 1 minute of the injection; this was followed by a period of segmental tremblings, involving some ventralward contraction (the animals lying on the side), and within 5 minutes after injection there appeared a very striking reversal of the behavior of the prolegs. Normally, and especially, in their natural use, when the creature creeps on the edge of a leaf, slight tactile stimulation of the ventrum between the legs suffices to induce the extension of

the terminal combs of the prolegs, simultaneous extension of the proleg pair, and their apposition—the whole movement giving an effective embrace of a leaf margin. The muscles governing the extension of the terminal combs, those having to do with the movements of the prolegs themselves, and the muscles of the body wall,[13] all are involved in this coördinated movement of response to stimulation of the ventral body wall; and under atropine the movement is entirely reversed — combs are retracted, prolegs greatly retracted, body wall itself retracted rather than, as normally, protruded in the region between the proleg. This "reversal" was found only in connection with the prolegs, never with the thoracic legs.

Normally, lightly touching a proleg comb, or the skin between the prolegs, leads to extension of both members of the pair; then a subsequent light touch leads to the usual clasping motion; under atropine, touching comb or skin between prolegs results in the spreading apart and deep retraction of the prolegs. The reversal of the proleg response persisted for 20 hours or more.

Atropine is very effective in producing heightened irritability; and usually there was evidence, also, of a tendency to dorsal contracture—slight suggestion of an opisthotonic state (perhaps related to the lifting of the head when the normal animal is disturbed on a leaf).

In one or two cases transitory evidence of proleg reversal was obtained during observation of larvæ injected with nicotine solutions, and particularly with pilocarpine; it could not be produced at will with these substances, however, nor was trace of the phenomenon detected under treatment with a variety of other neurophil drugs. It is a characteristic and pronounced effect peculiar, in my experience, to atropine.

With strychnine, and less clearly with atropine, suggestion was had of the special stimulation of the dorsal muscles ("opisthotonus"). This effect is not specific, or at least is not so specific as the proleg reversal. Tetraethylammonium chloride ($m/64$) invariably led to striking opisthotonic spasms, due chiefly to contraction of dorsal muscles at the thoracic level. Chloroform (one third saturated) behaved somewhat in the same way, but more

[13] Cf. Peterson, A., *Ann. Ent. Soc. Amer.* Vol. V., p. 246, 1912.

vaguely. Ventralward contraction, especially at the anterior end, was always produced by nicotine, camphor, phenol, resorcin, and caffeine.

Chemical Differentiation.

It is of interest to make note of certain facts bearing upon the question of phylogenetic neuromuscular differentiation, from the standpoint of reactivity to drugs.

The larvæ used agree in showing that the following instances exert a very definite neuromuscular excitation—evidenced by spontaneous writhings, often of definite pattern, or by temporarily heightened responsiveness, or both:

* nicotine ..... (1:500)
* picrotoxin ..... (1:240)
* camphor ..... (saturated)
* pilocarpine ..... (1:150)
* adrenaline ..... (1:1000)
phenol ..... (1:2000)
* resorcin ..... (1:1000)
caffeine ..... (saturated solution; 1:200, without effect)
* tetraethylammonium hydroxide (*m*/64)
chloroform ..... (1/3 saturated)
* atropine ..... (1:500)
strychnine ..... (1:100)

* (Especially exciting.)

Creatine, *m*/8, gave only slight temporary paralysis, probably not specific.

These facts suggest obvious differences when the behavior of the caterpillars is compared with that of other invertebrates. The action of atropine, and the relative lack of effectiveness of strychnine, point to parallelisms with conditions in cœlenterates,[14] rather than with forms closer akin. Crayfish injected with strychnine fail to yield evidence of "reversal" in the use of muscle groups, and atropine has a marked effect in stimulating the thoracic ganglia. But in how far the peculiarities here revealed are arthropodan characteristics cannot as yet be said.

In comparing a series of larvæ, such as the *Samias* and *Io*, one is struck by the fact that the *Io* caterpillar, provided with urticant

[14] Moore, A. R., *Proc. Nat. Acad. Sci.*, Vol. III., p. 598, 1917.

spines, shows normally a rather violent, jerky response to even mild stimulation. This is obviously a point of some significance, ethologically, since it increases the effectiveness of the "hairs" in penetrating and stinging. But under appropriate drug injection the quick, "snappy" type of behavior rather peculiar to *Io* can be easily brought about, for example, in a *Samia* caterpillar. Pilocarpine gave especially instructive comparisons of this sort. The sensory thresholds are so lowered by this substance that a *Protoparce* or *Samia* twists sharply in response to a single light touch, contracting at the place of stimulation, just as *Io* normally does. Biting reactions are likewise enhanced, so that *Samia* snaps savagely at its own spines encountered in writhing, or at forceps touching the head, much as *Io* normally does. The production of behavior normal to one species in another form characteristically more sluggish shows that the drug produces no *new* forms of response, but merely accentuates types of reaction for which the structural pathways already exist. Some light is thus given on structural basis of behavior differences in related forms.

The action of atropine shows clearly that reciprocal innervation exists in these insects, at least so far as concerns the action of the antagonistic muscle groups of the prolegs and walls of the segments. That strychnine, even in high concentration, fails to react in its characteristic manner with the synapses necessarily involved in these reciprocally acting nervous elements, shows that strychnine may fail to reveal the presence of synapses; and also that synapses even in types so closely related as annelids and arthropods may differ from one another;[15] or else that the "reversal" phenomena induced by atropine are brought about through action of the latter upon a locus distinct from that usually reacting with strychnine.

[15] Cf. Cushney, A. R., *Science*, N.S., Vol. XLIV., p. 482, 1916. It may be suggested that the slight acidity of caterpillar hæmolymph (p_H 6.8–6.6; Jameson and Atkins, *Biochem. Jour.*, Vol. XV., p. 209, 1921) might influence the action of the drugs; but I doubt that the low toxicity, at least, of strychnine can be explained in this way.